acids,[2] and the stereostructure presented in Fig. 1 represents the absolute configuration of omphalotin. Although it has not yet been proved, it is reasonable to assume that omphalotin is the biogenetic precursor of the oxidised omphalotins (B, C and D).[2] In fact, the omphalotins B, C and D always appear after omphalotin during fermentations.

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Evidence of an Attractant from Virgin Females of *Bephratelloides pomorum* (Hymenoptera: Eurytomidae): Possible Role of Cuticular Compounds

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Abstract: The Annona seed borer, *Bephratelloides pomorum* (Hymenoptera: Eurytomidae), is the most important insect pest of soursop, *Annona muricata* L. The female lays eggs directly into the most recently formed seeds of developing fruit where the larvae remain feeding until pupation. When fully developed, the young wasps make a channel to emerge from the fruit, ready to copulate. Males are attracted to females and display a peculiar behaviour which consists of three steps: antennation, lateral bouncing and wing vibrations. Experiments carried out in semi-field and laboratory conditions showed that males also behave similarly in the presence of filter paper impregnated with a hexane extract of the female's body, suggesting that female *B. pomorum* produce an attractant which enables males to find them. The hexane extracts of females, analysed by GC-MS, were shown to consist mainly of a mixture of straight- and branched-chain hydrocarbons and esters.

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Key words: *Annona* sp.; *Bephratelloides* sp.; GC-MS; attractants

1 Introduction

In the north-east of Brazil, the Annona seed borer *Bephratelloides pomorum* (Hymenoptera: Eurytomidae) is the most important insect pest of soursop, *Annona muricata* L. The female oviposits into the most recently formed seeds of developing fruit, which serve as food for the larvae. When fully developed, the young wasps start to excavate an escape tunnel towards the surface of the fruit; this may also result in indirect damage, since fungi and bacteria may invade the fruit through the escape holes made by adult wasps. As a result some attacked fruits fail to develop, they darken, mummify and drop.[1,2] Several authors have reported that wasps of the genus *Bephratelloides* are able to reduce fruit production by more than 70%.[3]

In some species of parasitic wasp, such as *Apanteles* (=*Cotesia*) *liparidis*, and *Cardiochiles nigriceps* Vier., females are responsible for the production of sex pheromone, which helps males to find them.[4,5] However, very little is known about the biology and mating behaviour of *B. pomorum*. Therefore, we decided to study the mating behaviour of wild *B. pomorum* in order to understand the mechanisms involved in the reproductive behaviour of this species.

2 Materials and methods

2.1 Insects. Wild adult wasps of *B. pomorum* were obtained from infested soursop fruits from a commercial orchard in Maceió, Alagoas (north-east Brazil). The fruits were taken to the laboratory and kept in wooden cages until adult emergence. The adults were segregated by sex and kept in separate cages until used.

2.2 Field-cage experiments. The mating behaviour of *B. pomorum* was observed in a field-cage (2 m^3) made from 20-mm nylon mesh. One potted host tree (height 1·6 m) was placed inside the field cage and two infested soursop fruits were hung from branches using cotton string. The fruits were replaced every three days. The average conditions of temperature and relative humidity inside the field-cage were 26($\pm$2)°C and 80($\pm$10)%, respectively. The experiment was replicated 10 times. For each replicate, 20 newly emerged male *B. pomorum* were taken from the wooden holding cages, marked on the thorax with one or two small spots of a non-toxic coloured paint (without anaesthesia) and then released into the field-cage. Their behaviour was then monitored over 90 min.

To observe the attraction of virgin male *B. pomorum* to virgin females, two plastic cages, made from recycled Coca-Cola® bottles, were used. One cage was painted with a black dye and its surface was perforated with small holes to resemble an attacked soursop fruit. The second cage was colourless and transparent and non-

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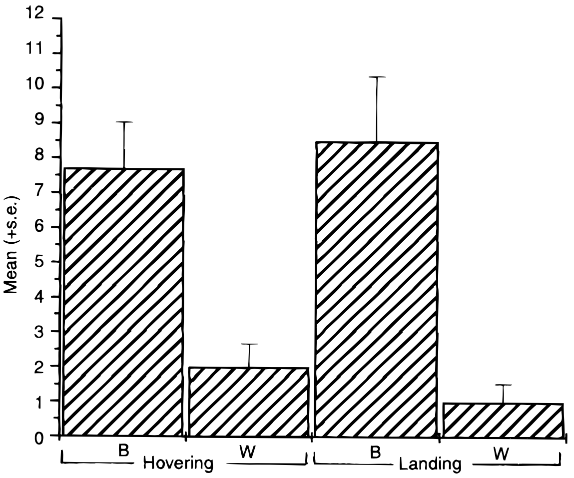

Fig. 1. Mean number of male *Bephratelloides pomorum* that were attracted to (B) black perforated cages and to (W) transparent non-perforated cages, each containing 20 females. Observations were carried out for 90 min. Means differ ($P < 0{\cdot}05$) using Tukey's test.

perforated. Both cages were hung on the branches of the potted tree using cotton strings. Thirty newly emerged males were released into the field-cage. The number of males attracted to each cage was counted and their behaviour was observed. This test was replicated 10 times.

In a further experiment, both uncoloured and black cages were perforated and no females were placed inside them in order to observe whether male *B. pomorum* are attracted to plastic cages only because those with holes resembled the surface of a soursop fruit. The procedure was the same as described above.

2.3 Laboratory experiment. Pieces of filter paper were impregnated either with an extract (30 μl) made by

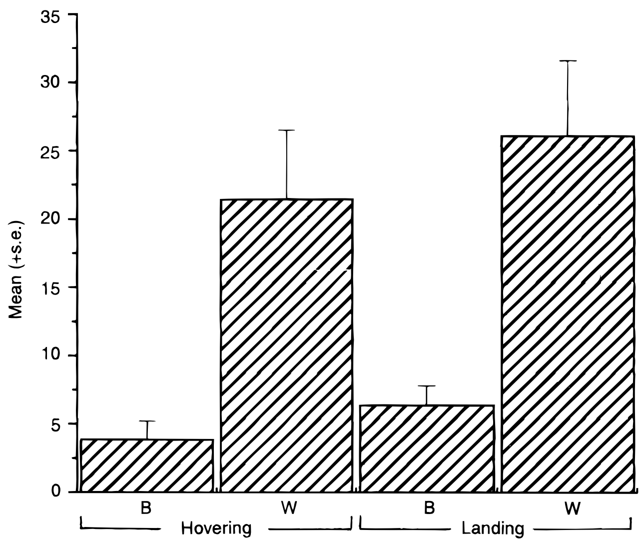

Fig. 2. Mean number of male *Bephratelloides pomorum* that were attracted to (B) black perforated cages and to (W) transparent perforated cages in the absence of females. Observations were carried out for 90 min. Means differ ($P < 0{\cdot}05$) using Tukey's test.

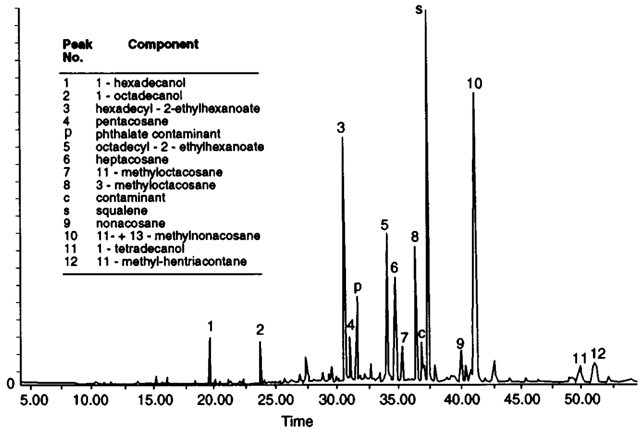

Fig. 3. Total Ion Chromatogram showing the compounds present in the extract obtained by washing whole bodies of virgin female *Bephratelloides pomorum* with hexane.

washing the head, thorax or abdomen of a virgin female seed borer with hexane, or with an equal volume of hexane only, and one piece of each type was placed at opposite corners of the interior of a clear plastic lunch box (22 × 22 × 5·5 cm). Ten virgin male seed borers were then introduced into the box and their behaviour observed over a period of 10 min. This procedure was replicated.

2.4 Chemical analyses. Extracts obtained by washing the whole body of virgin female *B. pomorum* with hexane (HPLC grade; 5 ml) were analysed by GC-MS using a Hewlett Packard 5890 gas chromatograph and 5870 Mass Selective Detector (MSD) with control by HP59970 C ChemStation. A fused silica capillary column (12 m × 0·32 mm I.D.) coated with methylsilicone gum of 0·33 μm film thickness was used with helium as carrier gas at 80 psi column head pressure. The column oven was programmed from 100°C for 2 min, then increased 5°C min^{-1} to 250°C. The injections were splitless. The mass spectrometer was set at 70 eV ionization energy and scanned from *m/z* 35 to 550 in the Scan mode under Autotune conditions.

3 Results and discussion

Observations of the mating behaviour of *B. pomorum*, performed in semi-field and laboratory conditions showed that, as soon as the emerging wasp reaches the surface of the fruit, it is ready for mating. Males usually emerge first and await the emergence of a female. Recognition of a female occurs at close range and attracted males display a peculiar courtship behaviour which can be divided into the three steps antennation, lateral bouncing and wing vibrations.

Ethological tests carred out in the laboratory with hexane extracts of head, thorax or abdomen, showed that the thorax is the most active region of the female's body. However, we have not yet found any source of pheromone production (e.g. exocrine gland) in this region, but morphological studies are being carried out.

Tests showed that males are more attracted to virgin females in the black perforated cages than to those in the transparent non-perforated cage (Fig. 1). This finding suggests that compounds released by females are responsible for the attraction of males. It has also been shown that the visual effect appeared not to play an important role in the attraction of males.

Figure 2 shows that the attraction of males to the white cage without females was due to a positive phototaxis shown by these insects.

Chemical analyses of the hexane extract of virgin female *B. pomorum*, by GC and GC-MS, revealed that it is mainly composed of a mixture of straight- and branched-chain hydrocarbons (C_{25}–C_{30}) and the two esters, hexadecyl 2-ethylhexanoate and octadecyl 2-ethylhexanoate (Fig. 3). In ethological tests carried out in the laboratory with virgin male *B. pomorum*, the hexane extract proved to be active. Males displayed courtship behaviour (antennation, lateral bouncing and wing vibrations) in the presence of a filter paper impregnated with hexane extract. In addition, electrophysiological studies, performed with live virgin males, have shown that male antennae give a positive response to hexane extracts of virgin females. These findings strongly suggest that female *B. pomorum* produce an attractant which enable males to find them.

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Influence of Formulants and Salt Formation on Volatilisation and Activity of Pyrimethanil

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Abstract: Pyrimethanil is an anilinopyrimidine fungicide which is highly effective against grey mould (*Botrytis cinerea*) in many crops. Some of its effectiveness can be attributed to localised vapour-phase activity in parts of the canopy not reached by foliar spray. Control of pyrimethanil volatilisation was investigated using matrix-forming materials or by formation of salts using organic acids. Whilst matrix formation had little effect, organic salts of pyrimethanil showed much reduced volatility. Some of the salts showed different biological potential against cereal pathogens that were not well controlled by parent pyrimethanil. In addition, salt formation offered the possibility of significant changes to the physical form of pyrimethanil, opening up new formulation and coformulation opportunities.

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Key words: pyrimethanil; volatility; matrix; salts; formulation

1 *Introduction*

The anilinopyrimidine fungicide, pyrimethanil, offers highly effective control of grey mould (*Botrytis cinerea* Pers. ex Fr.) in a variety of crops.[1] Some of the activity can be attributed to vapour-phase effects, as the compound has a vapour pressure of 2·2 mPa, and, as the molecule is readily metabolised, such external redistribution can offer protection to new growth.

It was postulated that control of vapour release may increase the window of activity, and may give enhanced activity against pathogens against which this molecule is less effective. For example, activity against cereal pathogens is minimal, this being largely attributed to rapid metabolism subsequent to foliar penetration. Maintaining a surface matrix for controlled release and minimised penetration might prolong activity, particularly since studies by other workers have shown that it is possible to reduce the rate of vapour release of chlorpyrifos by using matrix-forming materials in controlled-release emulsions.[2]

2 *Materials and methods*

In order to evaluate the influence of matrix-forming materials on volatility, a range of esters was selected for evaluation for this purpose, including cetyl palmitate, stearyl palmitate, distearyl phthalate, pentaerythritol tetrastearate, myristyl myristate, synthetic spermaceti, sorbitan monopalmitate and PEG600 distearate. Their melting points range from 38 to 61°C. Abietic acid was also evaluated.

In addition to evaluating such matrix-forming materials, a selection of air-polymerising linseed oil derivatives of varying viscosity was also evaluated. As with the matrix-forming materials, the linseed oils were evaluated at 0·5, 1·0 and 2·0 g litre^{-1}. Pyrimethanil was used at 0·5 g litre^{-1} in all treatments. The various treatments were made up in toluene solution; pyrimethanil was co-dissolved with the various matrix materials or oils and [^{14}C] pyrimethanil was used to facilitate monitoring of loss. All treatment solutions were applied to glass cover slips which were randomly positioned in a controlled-environment room. Up to five replicates per

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